

INHERITANCE AND PHYSIOLOGY OF EFFICIENCY IN IRON UTILIZATION IN SOYBEANS¹

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INTRODUCTION

STRIKING differences in chlorosis typical of iron deficiency were noted in 1938 among a considerable number of soybean varieties when tested on calcareous soils for the first time since their introduction into the United States from Manchuria. Because these strains were not greatly different morphologically, it was thought that such wide differences in chlorosis would lend themselves especially well to a study of the inheritance and physiology of iron availability in plants.

Reported cases of significant differential responses of diverse plant genotypes within the same species to varying nutrient levels are not numerous. The more important to date have been reviewed recently by BURKHOLDER and McVEIGH (1940) and will not be discussed here. They largely concern differential response of inbred lines and hybrids of corn to varying increments or sources of the major growth elements.

No previous report has been found of intraspecific heritable differences in the hydrogen-ion concentration of vegetable plant tissues. Genetically controlled differences in the acidity of flower petal sap of *Primula sinensis* and other species have been reported by SCOTT-MONCRIEFF (1936, 1939). Macerated petal tissue of plants homozygous for the single recessive gene causing low acidity showed a pH of approximately 6.0 while the dominant allele resulted in a flower petal pH of approximately 5.3.

Although numerous investigations have been conducted on the availability of iron to plants, the problem is still unsolved in several aspects. Chlorosis due to iron deficiency has frequently been reported when plants were grown in either soils containing an excess of lime or nutrient solutions with high pH. Most early investigators assumed that the low hydrogen-ion concentration in either case caused iron to be precipitated in the medium, thereby rendering it non-available to plants. Discoveries that in many cases chlorotic tissues contained as much or more iron than normal tissues have altered the concepts of iron availability. Since iron may occur in abundance in tissues of chlorotic plants, it would seem that much or all of this iron is in non-available form, suggesting that some internal factors may be involved in its availability to chlorophyll-containing cells.

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Growth of *Zea mays* and other species, when plotted against pH of full nutrient solution media to which iron had been added in an inorganic form, was found by OLSEN (1935) to follow a bi-modal curve. Plants grown in solutions held at pH 4.5 or 8 developed no chlorosis, but those grown in solutions of pH 6 or 7 exhibited severe iron deficiency symptoms. Solution analyses revealed decreasing soluble iron with increasing pH until a minimum was encountered at pH 6. Corn tissue analyses revealed larger amounts of iron in chlorotic (pH 6 and 7) than in normal cultures (pH 4.5 and 8). OLSEN concluded that although adequate iron was absorbed in all cultures, at pH 6 and 7 considerable phosphate ions were also absorbed, resulting in precipitation of iron as ferric phosphate within the vascular bundles. At pH 8 a large portion of the phosphate was precipitated in the solution as calcium phosphate, thereby rendering phosphate concentration low in the tracheal sap resulting in adequate availability of iron. OLSEN contended that at pH 4 and 5, corn plants normally absorb less phosphate than at a higher pH, and, furthermore, as numerous investigations have shown that pH of the growth medium influences pH of cell sap to some extent, the sap was adequately high in hydrogen-ion concentration to prevent precipitation of iron. Later work supported these conclusions. No chlorosis occurred in media with pH from 4 to 8 when phosphate was restricted. With abnormally high phosphate, chlorosis occurred at the highest pH as well as at pH 6 and 7. OLSEN surmised that in the latter case phosphate was added in excess of calcium in the solution which permitted absorption of adequate quantities to precipitate the iron in the tracheal vessels. Low calcium content of plants grown under these conditions substantiated this viewpoint.

MATERIALS AND METHODS

Source of genetic material

From approximately 20 soybean introductions from the Orient by the DIVISION OF PLANT EXPLORATION AND INTRODUCTION, U. S. DEPT. OF AGRICULTURE, that exhibited iron deficiency symptoms, six were selected for this study. The efficient types were pure lines of four standard varieties that exhibited chlorosis only on soils containing above 40 percent calcium carbonate.

Crosses were made reciprocally in all possible combinations in 1939 and 1940 between and among the four efficient and six inefficient varieties, and backcrosses were made to the inefficient parents. The F_1 , F_2 , F_3 , and backcross generations of these crosses were grown in the greenhouse and in the field and the classification of their efficiency in iron utilization determined in a special nutrient solution described below.

Media for genotype classification

Development of a growth medium in which proper concentration of available iron could be maintained to give the differential response exhibited on calcareous soils proved difficult. Classification of efficiency types was not found feasible in highly calcareous soils in the greenhouse during the period of low light intensity encountered throughout the winter. Deposition of calcium carbonate in high lime soils of Iowa is highly variable, and no degree of hand mix-

ing rendered this soil sufficiently homogeneous in available iron to permit classification in the early stages of development.

Use of quartz gravel, sub-irrigated with nutrient solution, was attempted. Neither partial removal with acids of iron naturally present in crushed quartz nor complete removal with subsequent addition of magnetite proved suitable under the conditions of these experiments.

Nutrient solutions seemed to offer greater homogeneity than any other growth medium. In full nutrient solutions, however, adequate amounts of available iron could not be maintained for normal growth of even efficient varieties without maintaining the solution at a pH well below 6. Since nutrient absorption caused the pH of solutions to fluctuate greatly, this method was considered impractical.

Preliminary studies had shown that when phosphorus was restricted in the nutrient solution no iron deficiency symptoms occurred even though the pH remained at approximately 7.4. Furthermore, it was found that when an excess of iron tartrate was added to the solution, phosphate could be added in increasing amounts until the concentration of available iron was such that the iron efficient varieties were normal in every respect while the inefficient varieties exhibited severe chlorosis at the first trifoliate leaf stage. To maintain this differentiating level of available iron it was necessary to add small quantities of phosphate to the solution every few days. If no additional phosphate was added, the chlorotic plants slowly developed chlorophyll presumably because absorption of the phosphates in the solution rendered additional iron available. Since the differentiating level could be maintained readily, this medium was chosen for classification of genotypes. SMITH'S (1934) nutrient solution was used minus KH_2PO_4 . Approximately one part per million of iron was added in the form of ferric tartrate. An initial addition of slightly more than one part per million of the phosphate ion established the desired differentiating level of available iron. The solution was replenished with approximately one-fifth part per million of phosphate every two days to maintain the desired level of available iron.

Classification of genetic material

Seeds for the F_1 , F_2 , F_3 , backcrosses, and parents were germinated in gravel, and, after emergence, the seedlings were transferred to nutrient solution tanks. The extent of differentiation in chlorosis among the parents was used as a criterion for establishing the appropriate level of available iron. All material was inspected at least three times at two-day intervals and classified according to iron deficiency symptoms as evidenced by chlorosis. Each plant was assigned a score ranging from zero (dark green) to five (bleached yellow appearance to complete necrosis of the leaf and death of the growing point).

Sampling for compositional differences between parents

In the study of compositional differences between the parents it was considered advisable to grow these varieties in nutrient media under conditions which would promote optimum growth for both types. A moderate degree of

chlorosis was allowed to develop in the first trifoliate leaf of the inefficient varieties to prove their genetic purity. When approximately 4 ppm of iron in the tartrate form was supplied, all plants developed normal chlorophyll, and subsequent leaves showed no iron deficiency symptoms. Phosphate was added frequently in very small quantities, and it was presumed an adequate amount was available, since no plants exhibited nutrient deficiencies.

When the plants attained a height of approximately 14 inches two replications were removed from the tank, and the four plants of each variety, which constituted an experimental unit, were separated into stem and leaf samples. The leaves were rapidly divided into two samples by cutting each leaflet longitudinally with nickel plated shears. Stems were cut into one-half-inch lengths, mixed thoroughly, and divided into two samples. One of the two leaf and stem samples was immediately placed in a small, tightly stoppered vial and frozen on a layer of dry ice. The other sample was dried at 125°C.

Several hours later the frozen samples were removed as needed, the vials immersed in tepid water for several minutes, and the composite tissue fluid extracted in a small plant press constructed of low-iron brass. The fluid was caught in a glass vial and the desired amount transferred to the small cup of a glass electrode attachment to a Coleman 3C pH electrometer suitable for making determinations in micro-quantities of liquids. After the pH was determined, one ml of the filtered fluid, or a known portion of one ml, was pipetted into a Pyrex test tube for subsequent determination of soluble iron.

The oven dried samples were ground with a mortar and pestle, again placed in the drying chamber for several days, and stored in a desiccator until total iron determinations were made.

The remaining two replications in the nutrient solution tank were harvested two weeks later and prepared for analysis as previously described.

Analytical procedure

The tissue fluids (1 ml) and dry tissue samples (0.5 gm) were analyzed for iron using the o-phenanthroline method as described by SAYWELL and CUNNINGHAM (1937). Deviations from their technique consisted only in choice of acids for wet ashing, method of determining color intensity, and o-phenanthroline solvent used. One ml concentrated H_2SO_4 and one-half ml concentrated HNO_3 were used for digestion of organic matter. All excess HNO_3 was removed by boiling. Reduction of the ferric iron was accomplished with an excess of hydroxylamin-hydrochloride. One ml 0.10 percent aqueous solution of o-phenanthroline was subsequently added. The sample was then diluted with two ml of distilled water to diminish the violence of subsequent neutralization with concentrated NH_4OH . Congo red paper was used as an indicator. The solution was made up to 10 ml volume in the case of stem samples and 50 ml volume in the case of leaf samples.

Intensity of the red ferrous o-phenanthroline complex was measured with an Evelyn photo-electric colorimeter. A filter was used which had a transmission maximum at approximately 7080 Å°, found to be the maximum absorption band of the ferrous o-phenanthroline complex by FORTUNE and MELLON

(1938). The absorption curve was determined by galvanometer readings for standard iron solutions of various concentrations when plotted against parts per million of iron. This enabled direct transposition of galvanometer readings of samples with unknown iron content to parts per million.

The ground, dried leaf tissue was also analyzed for total potassium. The procedure employed was the modified sodium cobaltinitrite method as described by BROWN, ROBINSON, and BROWNING (1938). Sample size and method of digestion were the same as used for the determination of total iron. Percentage of potassium was calculated on a dry weight basis.

RESULTS

Parental differentiation on calcareous soil

To test the magnitude of differential performance of the five efficient and six inefficient varieties, four seedlings of each variety were transplanted into

TABLE 1

Analysis of variance of dry top weights of four efficient and six inefficient soybean varieties when grown in normal and calcareous soil.

SOURCE OF VARIATION	D F	MEAN SQUARES	
		NORMAL SOIL	CALCAREOUS SOIL
Reps.	2	6.89	0.07
Varieties:			
Eff. vs. Ineff.	1	9.19	34.35*
Within Eff.	3	4.20	0.33
Within Ineff.	5	2.20	0.25
Error	18	2.302	0.188

* F value exceeds the 1 percent point in level of significance.

pots of normal soil and soil obtained from a naturally occurring calcareous area containing approximately 30 percent calcium carbonate and having a pH of approximately 7.3. These treatments were replicated three times. Extreme chlorosis occurred in the inefficient plants when grown on the high carbonate soil, while no deficiency symptoms occurred in the efficient genotypes.

The analysis of variance for dry weight of tops at four weeks after transplanting is given in table 1. Differences within the efficient and the inefficient varieties on either calcareous or normal soils were not significant. Although on normal soils the mean dry weights of the two groups were not significantly different, the differences when grown on calcareous soil were highly significant.

Inheritance of efficiency in iron utilization

As previously described, genotype classification in the inheritance studies was based on the degree of chlorosis exhibited by the seedlings when grown in a nutrient solution which had been rendered low in available iron.

Although the testing of progenies extended throughout periods of low and adequate light intensities, as encountered throughout the winter and spring seasons, overlapping of classification of the efficient and inefficient parents occurred infrequently. Therefore, all tests of plants with comparable genetic constitution were grouped regardless of the period of testing.

Performance of parents

The performance of all parental varieties which were included in the various tests is summarized in table 2. The average chlorosis grade of the efficient parents was 0.09, while the mean of the inefficient parents was 3.78. Only one plant of the efficient parents was assigned a chlorosis grade above 1, and only 8 inefficient parent plants were given a grade below 2. Consequently, if the genotypes of these plants had not been known, and if all plants assigned chlorosis grades of 0 and 1 had been considered efficient genotypes and plants

TABLE 2
*Summary of performance of efficient and inefficient parents
when tested on low-available-iron media.*

VARIETY	CHLOROSIS GRADE						MEAN GRADE
	0	1	2	3	4	5	
Efficient:							
1 (Dunfield)	26	—	—	—	—	—	0.0
2 (Mandell)	20	3	1	—	—	—	0.2
3 (Illini)	30	2	—	—	—	—	0.1
4 (Mukden)	38	4	—	—	—	—	0.1
Total	114	9	1	—	—	—	0.09
Inefficient:							
5 (FPI. 54619-5-1)	—	—	3	1	8	19	4.4
6 (FPI. 88508)	—	—	3	7	9	2	3.5
7 (FPI. 88358)	—	—	—	—	6	9	4.6
8 (FPI. 88294)	—	2	3	20	19	25	3.9
9 (FPI. 87617)	—	4	9	18	24	15	3.5
0 (FPI. 88354)	—	2	7	8	7	6	3.3
Total	—	8	25	54	73	76	3.78

assigned grades of 2 to 5 inefficient genotypes, nine plants out of 360, or one out of 40 would have been classified erroneously. The frequency of error in genotype classification in segregating progenies was probably of this magnitude.

Performance of F_1 crosses

The frequency distribution of F_1 crosses within the six chlorosis grades in comparison with that of the parents appears in table 3. In this classification grades 0 to 1 included plants in which no definite iron chlorosis appeared, and grades 2 to 5 included plants showing different degrees of iron deficiency symp-

TABLE 3

Frequency distribution of chlorosis grades of parents and various F₁ crosses when grown in media low in available iron.

GENETIC MATERIAL STUDIED	CHLOROSIS GRADES							
	0	1	TOTAL 0 TO 1	2	3	4	5	2 TO 5
Eff. Parents	114	9	123	1	—	—	—	1
Ineff. Parents	—	8	8	25	54	73	76	228
Eff. × Eff. F ₁	44	3	47	—	—	—	—	0
Ineff. × Ineff. F ₁	—	1	1	6	14	23	86	129
Ineff. × Eff. F ₁	87	5	92	—	—	—	—	0

TABLE 4

Numbers of efficient and inefficient plants in F₂ populations resulting from crosses between efficient and inefficient varieties.

CROSS	EFFICIENT	INEFFICIENT	CHI-SQUARE* (3:1)
1 × 5	45	14	0.05
1 × 6	37	17	1.21
1 × 7	38	21	3.53
1 × 8	40	15	0.15
1 × 9	41	12	0.16
1 × 0	33	12	0.07
2 × 5	38	16	0.65
2 × 6	38	15	0.31
2 × 7	26	9	0.01
2 × 8	38	14	0.10
2 × 9	36	10	0.26
2 × 0	43	10	1.06
3 × 5	40	15	0.15
3 × 6	44	10	1.21
3 × 7	43	14	0.01
3 × 8	41	14	0.01
3 × 9	41	14	0.01
3 × 0	41	12	0.16
4 × 5	21	8	0.10
4 × 6	30	12	0.29
4 × 7	35	11	0.03
4 × 8	26	13	1.44
4 × 9	38	15	0.31
4 × 0	13	4	0.02
Total all F ₂	886	305	0.69

* χ^2 for 1 D F at 5 percent level = 3.84.

toms. The frequency distributions have been grouped into these two major classes.

In all six efficient \times efficient crosses only efficient F_1 plants were obtained. The 14 F_1 crosses of inefficient \times inefficient types resulted only in inefficient progeny, indicating that all the inefficient varieties possessed a common gene or genes conditioning the inefficiency in iron utilization. F_1 plants in each of 14 crosses between inefficient and efficient varieties were as efficient in iron utilization as their efficient parents, indicating complete dominance of this type. The dominance of iron efficiency under field conditions on calcareous soil is illustrated in figure 3.

Performance of efficient \times inefficient F_2 populations

F_2 populations of all possible crosses between the four efficient and six inefficient parents were tested in a nutrient solution rendered low in available iron (table 4). The ratio of efficient to inefficient plants in the total for all F_2 populations did not deviate significantly from the ratio of a 3:1 hypothesis. Each cross was also tested for conformity to this ratio by the chi-square test and revealed close agreement. The difference between the four efficient and six inefficient varieties in efficiency of iron utilization seemed to be conditioned by a single major gene.

Performance of backcross populations

F_1 plants from five crosses between two inefficient and three efficient parents were backcrossed to their respective inefficient parents. The frequency distributions of the backcross populations in regard to iron utilization efficiency appear in table 5.

TABLE 5
Numbers of efficient and inefficient plants in backcross populations.

BACKCROSS	EFFICIENT	INEFFICIENT	CHI-SQUARE* (1:1)
8 \times (8 \times 1)	13	12	0.04
8 \times (8 \times 2)	32	29	0.02
8 \times (8 \times 3)	7	13	1.80
Total χ^2			1.86
Total plants	52	54	0.04
9 \times (1 \times 9)	24	22	0.09
9 \times (3 \times 9)	14	10	0.67
Total χ^2			0.75
Total plants	38	32	0.51
Total	90	86	0.09

* χ^2 for 1 D F at 5 percent level = 3.84.

χ^2 for 2 D F at 5 percent level = 5.99.

χ^2 for 3 D F at 5 percent level = 7.81.

None of the backcross populations differed significantly from a 1:1 ratio.



FIGURE 1.—Four efficient (left four pots) and six inefficient (right six pots) soybean varieties growing in soil with low available iron.

FIGURE 2.—Four efficient (left four pots) and six inefficient (right six pots) soybean varieties growing in soil with adequate available iron.

FIGURE 3.—Three plants of parent 8 (left), three 8×4 F₁ plants, and two plants of parent 4 when growing on calcareous soil.

FIGURE 4.—Three F₃ lines when grown in low-available-iron nutrient solution—homozygous inefficient (upper row), segregating (center row) and homozygous efficient (lower row).

Performance of F_3 lines

During the summer of 1940, F_2 populations from efficient \times inefficient crosses involving efficient parents 2 and 4, and inefficient parents 6, 8, 9, and 0, were grown to maturity in soil with adequate available iron. The progeny of a limited number of F_2 plants, selected at random from each cross, were tested to determine their efficiency in iron utilization when grown in nutrient solution containing a low level of available iron. Because of limited facilities, only ten F_3 plants were grown as a test of each F_2 individual. If a single factor difference is assumed, this small number of plants could result in erroneous classification of approximately seven percent of the segregating progenies. Performance of F_3 progenies appears in table 6. A segregating line grown by chance between apparently homozygous efficient and inefficient lines is shown in figure 4.

TABLE 6

Classification of efficient \times inefficient F_2 populations on the basis of their progeny performance in the F_3 generation when grown in media low in available iron.

CROSS	HOMOZYGOUS EFFICIENT	SEGREGATING	HOMOZYGOUS INEFFICIENT	CHI-SQUARE* (1:2:1)
6 \times 2	2	9	5	1.37
8 \times 2	3	7	7	2.41
9 \times 2	3	9	6	1.00
0 \times 2	3	8	5	0.50
4 \times 6	2	10	6	2.00
4 \times 8	6	5	4	2.20
4 \times 9	2	11	3	2.37
4 \times 0	3	10	5	0.67
Total χ^2				12.52
Total plants	24	69	41	4.43

* χ^2 for 2 D F at 5 percent level = 5.99.

χ^2 for 16 D F at 5 percent level = 26.30.

The proportion of homozygous efficient, segregating, and homozygous inefficient F_3 lines in none of the F_2 populations deviated significantly from the expected 1:2:1 ratio. Furthermore, the sum of the chi-square values for all populations, and the chi-square value calculated from the deviations of total plants in each class from the expected number were also non-significant. Distribution of types in the segregating lines is shown in table 7.

None of the population totals for segregating lines deviated significantly from the 3:1 ratio. The total chi-square, as well as the chi-square for the ratio of all efficient and inefficient plants in the segregating lines, also indicates conformity with the single factor hypothesis.

Maternal inheritance test

Since reciprocal crosses between efficient and inefficient parents were frequently made, the data were examined as to the possibility of maternal inheritance (table 8).

TABLE 7

Numbers of efficient and inefficient plants in F₂ segregating lines.

CROSS	EFFICIENT	INEFFICIENT	CHI-SQUARE* (3:1)
6×2	66	26	0.52
8×2	47	19	0.51
9×2	63	24	0.31
0×2	56	23	0.71
4×6	70	29	0.97
4×8	34	16	1.31
4×9	79	31	0.59
4×0	75	24	0.03
Total χ^2			4.95
Total plants	490	192	3.61

* χ^2 for 1 D F at 5 percent level=3.84. χ^2 for 8 D F at 5 percent level=15.51.

TABLE 8

Performance of F₁ and F₂ generations of reciprocal crosses.

TYPE OF PARENT		CHLOROSIS GRADES								CHI-SQUARE*
FEMALE	MALE	0	1	TOTAL EFF.	2	3	4	5	TOTAL INEFF.	(3:1)
Ineff. × Eff. F ₁		60	3	63	—	—	—	—	0	—
Eff. × Ineff. F ₁		42	2	44	—	—	—	—	0	—
Ineff. × Eff. F ₂		302	45	347	14	48	33	38	133	1.74
Eff. × Ineff. F ₂		451	68	519	26	62	51	34	173	0.00

* χ^2 for 1 D F at 5 percent level=3.84.

Absence of maternal inheritance was evidenced by the similarity in performance of reciprocal crosses in both the F₁ and F₂ generations.

Physiological study

Leaf and stem tissues of the four efficient and six inefficient varieties used in the inheritance study were subjected to certain chemical analyses as previously outlined.

The following analyses were made: (1) pH of composite tissue fluid expressed after freezing samples of leaves and stems, (2) soluble iron in one ml of composite tissue fluid from leaves and stems, (3) total iron in dried stem and leaf tissues, and (4) total potassium in dried leaf tissues. Mean values of the four replications for the four efficient and six inefficient varieties appear in table 9.

The criteria, for which means are presented in table 9, have been subjected to analysis of variance (table 10).

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TABLE 9

Mean pH and soluble iron values of composite tissue fluids, soluble and total iron of leaf and stem tissues, and total potassium of leaf tissues (dry weight basis) of four efficient and six inefficient soybean varieties.

VARIETY	LEAVES				STEMS		
	pH	SOLUBLE Fe (ppm)	TOTAL Fe (%)	TOTAL K (%)	pH	SOLUBLE Fe (ppm)	TOTAL Fe (%)
Efficient:							
1	6.27	6.28	.098	3.56	5.90	5.70	.016
2	6.20	6.70	.096	3.75	5.88	5.10	.015
3	6.18	7.50	.093	3.90	5.87	5.08	.014
4	6.12	6.53	.085	4.04	5.83	6.45	.015
Mean	6.19	6.75	.093	3.81	5.87	5.83	.015
Inefficient:							
5	6.51	4.15	.121	3.48	5.99	5.93	.019
6	6.43	4.98	.110	3.48	5.98	5.00	.019
7	6.37	6.73	.097	3.70	5.96	6.10	.016
8	6.50	5.58	.133	3.39	5.99	5.50	.017
9	6.37	6.20	.101	3.58	5.92	6.15	.017
0	6.43	5.68	.105	3.52	5.95	4.98	.018
Mean	6.43	5.68	.111	3.53	5.97	5.61	.018

TABLE 10

Analysis of variance of pH, soluble iron, and total iron of leaf and stem tissues, and total potassium of leaf tissue of ten soybean varieties.

SOURCE OF VARIATION	D F	MEAN SQUARES			
		pH	SOLUBLE IRON	TOTAL IRON	TOTAL POTASSIUM
Leaves:					
Replications	3	.0034	0.10	.000264	.1425
Varieties					
Eff. and Ineff.	1	.5520†	11.10*	.003271*	.8109†
Within Eff.	3	.0164	1.12	.000126	.1668
Within Ineff.	5	.0151*	3.81	.000742†	.0438
Error	27	.00584	1.519	.0001740	.09022
Stems:					
Replications	3	.0006	0.32	.000000	
Varieties:					
Eff. and Ineff.	1	.0920†	0.48	.0000616†	
Within Eff.	3	.0039*	1.39	.0000033	
Within Ineff.	5	.0028	1.14	.0000038*	
Error	27	.00118	0.810	.00000133	

* F value exceeds the 5 percent point in level of significance.

† F value exceeds the 1 percent point in level of significance.

The analysis of variance shows that efficient and inefficient varieties differed significantly in hydrogen-ion concentration of composite tissue fluid of both leaves and stems. Although significant differences were found in the pH of leaf tissue among the inefficient, and in the pH of stems among the efficient varieties, these differences were far less in magnitude than differences between the efficient and inefficient varieties. The F values for leaves and stems of the latter were, respectively, 12 and 10 times the value necessary for highly significant differences.

These differences were substantiated by pH determinations of composite leaf tissue fluid of the ten varieties when grown in the field on non-calcareous soil during the summer of 1940. Under these conditions the F value of the pH difference between the two types exceeded that of the one percent point in level of significance by five times.

Differences in total iron content of tissues closely paralleled differences in pH of tissue fluid. Inefficient varieties contained a surprisingly greater amount of total iron than the efficient varieties. Analyses for soluble iron showed a significantly larger quantity in the expressed sap of leaf tissue from the efficient type. Although soluble iron in the stems showed the same relationship, the differences were statistically non-significant. Total potassium was significantly greater in the efficient varieties, while varieties within the two groups did not differ significantly.

The gene conditioning greater efficiency in iron utilization thus seems to be expressed by its action in producing higher hydrogen ion concentration, higher soluble iron, higher potassium and lower total iron content of aerial tissues than its allele which, when in the homozygous state, conditions inefficiency in iron utilization. A study of the associations between these various compositional criteria was made by means of the covariance method of analysis as outlined by SNEDECOR (1940). The relative degree of association was considered a possible means of approaching the cause and effect relationship. The following associations in the leaf tissues were investigated: pH and soluble iron, potassium and soluble iron, pH and total iron, potassium and total iron, and pH and potassium. The stem analysis associations studied were pH and soluble iron, and pH and total iron. The resulting errors of estimate mean squares and adjusted mean values for the efficient and inefficient varieties appear in table 11. Mean squares from the analysis of variance and unadjusted mean values for the two types of varieties are included to facilitate comparison of adjusted and unadjusted values.

The mean squares of errors of estimate indicate a close association between the hydrogen ion concentration of the expressed juice and total iron within the leaf and stem tissues. Highly significant total iron differences between the efficient and inefficient varieties were rendered non-significant after due allowance was made for pH differences.

Similarly, potassium and soluble iron differences within leaf tissues became non-significant when pH variation was taken into account. Although adjustment for the potassium association did reduce soluble and total iron differences between the efficient and inefficient genotypes, the potassium content did not

TABLE 11

Mean squares of errors of estimate and adjusted mean values of efficient and inefficient varieties resulting from the analysis of covariance of various compositional criteria.

SOURCE OF VARIATION	D F	SOLUBLE IRON			TOTAL IRON			TOTAL K	
		UNADJ.	ADJ. FOR pH	ADJ. FOR K	UNADJ.	ADJ. FOR pH	ADJ. FOR K	UNADJ.	ADJ. FOR pH
Leaf Tissues:									
Varieties:									
Eff. and Ineff.	1	11.10*	0.23	3.02	.003271†	.000247	.000798*	.8109†	.0024
Within Eff.	3	1.12	1.21	0.95	.000126	.000034	.000022	.1668	.0445
Within Ineff.	5	3.81	2.02	3.05	.000742	.000168	.000475†	.0438	.0140
Error	27‡	1.519	1.372	1.38	.0001740	.000091	.000111	.09022	.08263
Variety Means:									
Efficient		6.75	5.91	6.49	.0928	.1103	.0975	3.81	3.62
Inefficient		5.68	6.24	5.85	.1112	.0995	.1080	3.52	3.65
Difference	-	1.07	-0.33	0.64	-.0184	.0108	-.0105	0.29	-0.03
Stem Tissues:									
Varieties:									
Eff. and Ineff.	1	0.48	1.04		.0000616†	.0000013			
Within Eff.	3	1.39	0.78		.0000030	.0000014			
Within Ineff.	5	1.14	1.02		.0000039*	.0000039†			
Error	27‡	0.810	0.743		.00000133	.00000097			
Variety Means:									
Efficient		5.83	5.30		.0152	.0162			
Inefficient		5.61	5.97		.0177	.0170			
Difference		0.22	-0.67		-.0025	-.0008			

* F value exceeds 5 percent point in level of significance.

† F value exceeds 1 percent point in level of significance.

‡ 26 D F for mean squares of errors of estimate.

seem to account for as much of the iron variation as was explained by the hydrogen-ion concentration differences.

DISCUSSION

In view of the complex nature of mineral absorption and utilization in plants, the monogenic simplicity of the genetic control of iron utilization efficiency discovered in these investigations seems remarkable. Although some variation of efficiency was noted among the inefficient varieties, the magnitude of expression of any modifying genes was negligible in comparison with that of the major gene involved. Designation of the recessive gene conditioning inefficiency in iron utilization by the symbol *fe* is suggested. The dominant allele conditioning efficiency would therefore be *Fe*.

Since the gene conditioning efficiency proved identical in the six varieties, and since 14 additional inefficient varieties probably carried this gene, an effort was made to trace the origin of varieties of this type. These varieties came from central and southern Manchuria or northern Chosen. Several of the inefficient varieties were collected from the same locality, but in all cases efficient varieties were also obtained from the same locality. No marked selection against the inefficient genotype seems to have occurred. This is especially odd since calcareous and alkaline soils of various kinds have been found by THORP (1936) to occur generally throughout central and southern Manchuria.

Since soybean varieties in the Orient are generally developed for local areas and normally do not spread rapidly, it is possible that the inefficient varieties were grown on upland soil from which lime had been leached, and no selection against this genotype occurred.

Chemical analyses of stem and leaf tissues were made to determine the physiological differentiation between the genotypes. Differences in hydrogen-ion concentration of expressed tissue fluid were most consistent of the constituents measured. Such differences were obtained not only when the two genotypes were grown in nutrient solutions but also when grown on normal soil under field conditions. The identification of the hydrogen-ion concentration of expressed cell sap with the true intracellular pH is open to criticism. As pointed out by OSTERHOUT (1922), expressed juice does not represent an unaltered cell sap, for, in crushing the cells, the sap may be changed by chemical reactions, absorption, or admixture with intercellular substances. ROGERS and SHIVE (1932) found that although various tissues within a plant differed greatly in hydrogen-ion concentration, the pH of expressed fluid was fairly constant and approximated the mean hydrogen-ion concentration of the various tissues. Furthermore, the pH of composite tissue fluid of various species was proportionate to the pH of similar tissues within the different species. The observed pH difference of the efficient and inefficient soybean varieties is therefore believed to be proportional to the intracellular pH difference of the two types.

Relatively lower solubility of the iron and lower potassium content was associated with the higher pH of the inefficient genotype. The interrelation of cell sap pH, total and soluble iron, and potassium content of plant tissues is not well understood although various associations have been reported. The investigations of INGALLS and SHIVE (1931) and ROGERS and SHIVE (1932) showed a definite relationship between the pH of expressed tissue fluid and the solubility and quantity of iron present within the plant.

HOFFER and TROST (1923) contended that excessive accumulations of iron in the nodal regions of the corn stem were often associated with inadequate supplies of available potassium in the growth medium, and consequently, in the corn stems. The converse interrelation of potassium and calcium absorption by plants, as found by LOEHWIN (1928), may be of significance. Media with high calcium resulted in high sap pH and copious precipitation of iron in root and lower stem regions, while a consistent decrease of pH in the expressed plant sap was associated with the addition of potassium to the growth medium. This relationship was considered responsible for a lack of iron deficiency symptoms accompanying the potassium treatment.

In view of these various findings it is suggested that the inefficient gene may condition lower potassium concentration which produces higher pH of cell sap and thereby causes the solubility of iron to be decreased. Although in this study potassium analyses were conducted as an afterthought when genotype differences in iron solubility were discovered, an investigation is planned to ascertain the comparable efficiency of the two genotypes when grown on various levels of potassium. The results of such an experiment coupled with tissue

analyses of iron and potassium should give further information on this complex cause-and-effect relationship.

SUMMARY

Marked differences in chlorosis were noted among soybean varieties when tested on calcareous soils for the first time since their introduction into the United States from Manchuria. Testing of these varieties in nutrient solution cultures and in sub-irrigated crushed quartz media proved that this differential performance could be induced when the plants were grown on media in which the concentration of available iron was low. When grown on such media, varieties which were efficient in iron utilization made normal, green growth, while inefficient varieties showed severe chlorosis symptomatic of iron deficiency which ultimately resulted in death of the plant. The difference between efficient and inefficient genotypes was of sufficient magnitude to permit classification when grown in media rendered low in available iron, which made possible a study of the mode of inheritance of efficiency differences.

On the basis of F_2 and F_3 populations of crosses between four efficient and six inefficient varieties, and of backcross populations, differences in efficiency of iron utilization were shown to be conditioned by a single gene. Inefficiency of F_1 plants from all crosses among the six inefficient varieties established allelism of the gene conditioning inefficiency, and the assumption was made that the inefficient gene was identical in these varieties. The recessive gene conditioning inefficiency in iron utilization was assigned the symbol *fe*.

Performance of F_1 plants from crosses between efficient and inefficient varieties indicated complete dominance of the *Fe* allele. Absence of maternal inheritance was evidenced by similar performance of reciprocal crosses between the *Fe* and *fe* genotypes in the F_1 and F_2 generations.

Composition in aerial plant tissues, as conditioned by the inefficient gene, consisted of relatively higher pH, lower soluble iron, higher total iron, and lower potassium content. Although the data furnished no definite information as to identity of the primary causal agent, the assumption was made that relatively low solubility of iron in inefficient genotypes was induced by the comparatively high pH. On the other hand, relatively low potassium content might have accounted for the low hydrogen-ion concentration.

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